Superoxide Dismutase (SOD) and Catalase (CAT) role in Plant Growth and Nitrogen fixing competence of *Vigna mungo* genotypes under Fluoride induced Oxidative Stress.

A Synopsis

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1. INTRODUCTION

Fluorine a halogen is 13th most exuberant element found in the earth crust (Singh and Roy Chaudhary, 2020). In numerous nations, the fluoride ion (F) contamination of ground water and soils is a major concern when it is present at levels over the authorised limits. The World Health Organization established a recommendation for a "desirable" upper limit of F in drinking water of 1.5 ppm (mg/L) in 1984, and it was reaffirmed in 1993. India (1 ppm) and Senegal (0.6 ppm) in West Africa have lower national guidelines for fluoride concentration, with the Indian restriction carrying the caveat that "lesser the fluoride the better, as fluoride is detrimental to health" (Yadu et al., 2016). Including India near about 200 million people from 29 countries are severely affected because presence of fluoride beyond permissible limits (Bhattacharya et al., 2018). Chronic fluoride (F) exposure has been shown to have harmful consequences on humans, domestic animals and plants. Many plant species have been examined and reported to have undergone physio-biochemical alterations as a result of Fluoride toxicity (Chakrabarti and patra, 2015).

Over the past few decades, inevitable and ongoing human activities including industrial emissions, mining, smelting, metal processing, agricultural practises, etc. have significantly increased the amount of metals and non-metals in the aquatic environment (EPA,1978). Fluoride is one of them. Rocks and volcanic ashes weathering naturally produces fluoride and are the natural sources of fluoride pollution. The most significant inorganic fluorides that have had negative effects on the environment include sodium fluoride, calcium fluoride, silico-fluoride, sulphur hexafluoride, and hydrogen fluoride (Sharma and Kaur, 2019).

Fluoride endemic areas frequently use groundwater tainted with fluoride for irrigation, which has a significant negative impact on crops. F^- suppresses germination, damages mitochondrial and chloroplast ultrastructure, affects membrane permeability, and reduces photosynthetic efficiency, all of which have an impact on plant growth, biomass, and productivity (Singh et al., 2013). Leaves can take up fluoride from the air and roots can take it up from the soil (Zhang et al., 2013). It reaches the mesophyll cells, which are the most productive photosynthetic zone, causing a reduction in the PS II's chlorophyll fluorescence yield (Fv/Fm) (Singh et al., 2013). Higher fluoride level creates stress condition in plants known as oxidative stress. An oxidative stress

is any disturbance in the cellular redox equilibrium that leads to the generation of reactive oxygen species (ROS) as a result of oxygen's univalent reduction. Through accelerated electron leakage to oxygen, salt stress speeds up the creation of ROS such superoxide radicals (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), alkoxyl radicals (RO), and singlet oxygen ($^1O_2$). The ability of these cytotoxic ROS, which are also produced during metabolic activities in the mitochondria and peroxisomes, to cause oxidative damage to lipids, proteins, and nucleic acids and so interfere with normal metabolism is already well established. Free radical-induced lipid peroxidation plays a significant role in the breakdown of membranes (Panda and Khan, 2009). Plant cells are equipped with a group of antioxidants that includes both enzymatic and non-enzymatic components that may defend the stressed cells under adverse conditions to battle abiotic stress, such as F-promoted ROS (Yadu et al.,2016). Some of the enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX), glutathione reductase (GR), monodehydroascorbate reductase, and glutathione-S-transferase. Ascorbic acid (AA), glutathione, proline (Pro), tocopherol, and flavonoids are examples of non-enzymatic antioxidants (Gill and Tuteja, 2010). One of the main obstacles to producing crops profitably is salt stress, which affects more than 800 million (ha) of land worldwide (FAO, 2008).

India is the world's top producer (25%), consumer (27%), and importer (14% of all pulses). One of the least expensive foods are pulses and are good source of protein in India for health. The World Health Organization advises the pulse consumption of 80 grams per person every day for India (Rajput and Arjun, 2020). Black gram is farmed in India on 3.07 million hectares of land, producing 1.60 million metric tonnes annually. In Rajasthan, black gram production is significantly lower than the national average (Jangir et al., 2016).

The two most prevalent nations with endemic fluorosis are China and India, with Rajasthan in India having the worse condition with fluoride pollution in all 33 districts. Due to the existence of fluoride-bearing rocks and minerals in the vicinity, this state has a large number of fluoride-affected places, with an average concentration of 16 mg/l. The principal fluoride-affected areas in the state include Ajmer, Alwar, Banaswara, Barmer, Bharatpur, Bhilwara, Bikaner, Jaipur, Dausa, Rajasamand, Hanumangarh, Pali, Nagur, Sirohi, and Ganganagar (Rai., 2021). In all

the mentioned districts *Vigna mungo (L.)* is one extensively grown crop. This shows that it is somehow resistant to fluoride stress. With a 50,089-ha area, 17,111 tonnes of yearly output, and an average yield of 342 kg/ha, Bhilwara holds the top spot for *Vigna mungo* cultivation (Seema Jat et al., 2017). It is mostly grown on marginal areas in southern Rajasthan's rain-fed mono/mixed cropping system (Jha et al., 2015). It contributes around 23.44% of total crop production of Rajasthan in 2019-20 (economic survey, 2021-22). Along with Madhya Pradesh, Maharashtra, Karnataka, and Uttar Pradesh, Rajasthan is a significant producer of pulses. Rajasthan planted pulses on a total of 4197.7 thousand hectares in 2013–14, producing 2490.9 thousand tonnes at a productivity of 593 kg/ha. A kharif crop moth's yield, which was 237 kg/ha in 1976–77, climbed to 339 kg/ha in 2011–12 showing the increase in pulse production of Rajasthan (Sharma and Bhushan, 2019).

Pulses are leguminous crops, because of their symbiotic relationship with soil rhizobia they have the capacity to fix nitrogen in the root nodules. They may be effectively utilised to improve salty soil fertility and aid to return agriculture to these places due to their ability to thrive on nitrogen-poor soils. However, salt stress significantly reduces the production of legumes due to its negative effects on the host plant's growth, the bacteria in the root nodules, the formation of symbiotic relationships, and finally the ability to fix nitrogen (Manchanda and Garg, 2008). About 80% of the atmosphere is composed of N_2, but it must first be converted to NH_3 in order to be digested. Nitrogenase is an enzyme that catalyse the conversion of N_2 to NH_3, and it is extremely sensitive to O_2 damage.

In the current study, we intend to investigate how the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) aid *Vigna mungo (L.)* 5 genotypes in coping with fluoride stress along with the symbiotic relationship, as well as which genotype is more competent to develop under stress and the involvement of nitrogenase in the competence.

2. REVIEW OF LITERATURE

Crop productivity is significantly hampered by environmental conditions as micronutrient deficiency, salinity, methyl viologen, drought, heat, chilling, freezing temperatures and chilling. The generation of reactive oxygen species (ROS), such as the superoxide radical hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), and singlet oxygen species ($^1O_2$), is accelerated by these stressors. These ROS may severely oxidise proteins, lipids, chlorophyll, and nucleic acids if they aren't detoxified (Gupta et al., 2009). Fluoride (F) contamination is well acknowledged as posing a major risk to the biotic elements of natural settings. It was found to impede the transfer of water and minerals, which are necessary for the physiological and biochemical functions of plants. By increasing the generation of reactive oxygen species (ROS) including superoxide (O^{2-}) and hydrogen peroxide, fluoride particularly disturbs the redox equilibrium of the cells (Yadu et al., 2018). The plants have created a sophisticated antioxidative defence mechanism to lessen the harm that ROS causes. The antioxidant defence system contains two major components (1) enzymatic antioxidants (2) non-enzymatic antioxidants. Carotenoids, ascorbate, glutathione, and tocopherols are components of the non-enzymatic antioxidative system. Enzymatic components include superoxide dismutase, catalase, glutathione peroxidase, peroxidases, and enzymes involved in the ascorbate-glutathione cycle, ascorbate peroxides, dehydroascorbate reductase (Panda and Khan, 2009).

The primary intracellular antioxidant defence against free radicals is considered to be SOD (Stephenie et al., 2019).

Superoxide dismutase is a catalyst that breaks down superoxide radicals into oxygen and hydrogen peroxide. SOD is an important defence against oxygen radical-mediated toxicity in higher plants. According to the metals present in the active site, three different SODs have been recognised: copper/zinc SOD, manganese SOD, and iron SOD. Higher plants have had FeSOD, MnSOD, and Cu/ZnSOD isolated from their plastids, mitochondrial matrix and cytosol, respectively. In the chloroplast of certain plants, there is even another isoform (Ray chaudhari and Deng, 2000). Manganese SOD (Mn-SOD) homo-tetramer isoform, which is primarily present in the matrix and inner membrane of mitochondria; copper-zinc SOD (Cu/Zn-SOD) homodimer isoform, which is present in the nucleus, cytoplasm, plasma, and inter-

membrane space of mitochondria; and Cu/Zn SOD (EC-SOD), a tetrameric glycoprotein isoform that is localised extracellularly (Stephenie et al., 2019).

The second enzyme involved in the enzymatic antioxidant defence system is Catalase. Thenard discovered catalase activity in plant and animal tissues for the first time in 1818. Catalase was initially isolated and crystallised from beef liver, and Sumner and Dounce substantiated its identification 1937 (Scandalios et al., 1990).

It is a homotetramer of around 250 kDa that contains heme and is present in all eukaryotes and prokaryotes. Catalase, an enzyme-based antioxidant, plays a crucial part in preventing cellular oxidative damage by efficiently converting hydrogen peroxide (H_2O_2) into water and oxygen (Kandukuri et al., 2012). Although it is mostly found in peroxisomes, this enzyme is also found in the mitochondria and cytoplasm of cells. In plants, catalase removes H_2O_2 produced by photorespiration under both normal and stressful circumstances, help in oxidation of fatty acids and mitochondrial electron transport. The existence of numerous catalase isoforms (CAT1, CAT2, CAT3) encoded by different genes (Cat1, Cat2, Cat3) expressed in organelles in temporal and stress-specific ways has recently been shown (Sharma and Ahmad., 2014).

The oxidative stress affects the productivity of legumes worldwide. Legume crops are important economically since they are consumed by millions of people worldwide and have exceptional nutritional qualities. Legume seeds provide a variety of nutrients that are good for human health, including fatty acids, folic acid, vitamins, minerals, and carbs (twice or treble the amount found in most cereals). The di-nitrogen (N_2)-fixing rhizobia are often found in legume nodules as organelle-like structures inside their root host cells. Numerous mechanisms, including as nitrogen (N)/carbon (C) metabolisms, oxygen transport through nodules, oxidative stress and phosphorous (P) levels, function and interact within the symbiotic interaction between plants and nodules. These procedures affect how N_2 fixation is regulated (Schwember et al., 2019). High salinity has an impact on plant development, particularly shoot growth, and therefore on the symbiotic relationships in legumes. Specific nitrogenase activity is inhibited, which is a direct reflection of salinity's detrimental impact on the Rhizobium-legume symbiosis. Oxidative stress effects many factors such as Leghemoglobin depletion and the amount of carbohydrates in the cytosol and bacteroids. (Pla and Cobos-Porras, 2015). The central step in the biological nitrogen

fixation process is the ATP-dependent reduction of dinitrogen to ammonia, which is catalysed by nitrogenase. Nitrogenase is a two-protein system that catalyses the hydrolysis of ATP and the reduction of dinitrogen to ammonia. The molybdenum-containing system, which consists of two component metalloproteins, the iron (Fe) protein and the molybdenum-iron MoFe-protein, where the Fe-protein is the nucleotide-binding and electron-donating component and the MoFe-protein contains the substrate-reducing site, is the most extensively studied form of nitrogenase (Rees and Howard, 2000).

Ahmad et al., 2012 findings demonstrated that salt stress greatly reduced mung bean growth, yield, and physiological indices, but that inoculation with either rhizobia or PGPR alone considerably improved these characteristics. However, the combination use of rhizobia and PGPR proved more successful in lessening the mung bean's lowering effects of salinity. The findings suggest that using Rhizobium and Pseudomonas strains together can increase mung bean production.

Aggarwal and Khan, 2016 investigated fluoride's effect on *Vigna radiata. Vigna radiata* seedlings were treated with NaF (0, 25, 50, 75, or 100 mg NaF kg^{-1}) and its impact on germination, growth, and RAPD profile was examined. The RAPD-PCR method was used to find the variation in DNA profile caused by NaF treatment.

Roychaudhury et al., 2021 The purpose was to investigate the differences in how *Vigna radiata* and *Vigna mungo (L.)* responded to fluoride stress (25 mg L^{-1} NaF) and to support the protective effects of sodium nitroprusside (SNP) (50 M). Compared to *V. mungo, V. radiata* had a bioaccumulation of fluoride that was greater. Overall, the research proved that the species *V. radiata* and *V. mungo* are, respectively, fluoride-sensitive and fluoride-tolerant, and it also demonstrated the effectiveness of SNP in reducing stress-induced damage to *Vigna* by enhancing the defence mechanism.

Jayakumar et al., 2008 examined how cobalt treatment affected the antioxidant enzyme activities of *Vigna radiata* (L.) Wilczek. After planting, biochemical parameters including reducing and non-reducing sugar, starch, amino acid, and protein content, as well as the activities of antioxidant enzymes including catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO), were analysed (DAS). The study seen significant impact on all parameters.

Cai et al., 2016 monitored photosynthesis, antioxidant defence, and cell ultrastructure at various F concentrations (0–50 mg L^{-1}) to better understand how tea responds to the stress imposed by F. Decreases in photosynthetic and chlorophyll fluorescence characteristics were brought on by high F (5 mg L^{-1}). F changed activated oxygen metabolism as seen by the rise in lipid peroxidation, electrolyte leakage (EL), and H_2O_2 build up. With rising F concentration, manganese superoxide dismutase (Mn-SOD, EC 1.15.1.1) activity decreased. Antioxidant enzymes measured activity matched up with how their genes were expressed. The findings imply that at 5 mg L^{-1} F but not at 20–50 mg L^{-1} F, the antioxidant enzymes in the tea plant may work the best in removing reactive oxygen species (ROS).

Benmoussa et al., 2022 The study examined the behaviour of six *faba* bean (*Vicia faba* Minor) genotypes (Saber 02, Locale, Baachar, Badii, Chourouk, and Najeh) in response to salinity and flooding (hypoxia), either alone or combined, to identify tolerant genotypes and choose effective faba bean-rhizobia symbiosis. 90 different bacterial strains were found in the nodules of the *Vicia faba* root. Four strains were chosen as the best *faba* bean symbiotic partners following the characterisation, identification, and selection processes based on their symbiotic effectiveness and salt tolerance behaviours.

Singh A et al., 2021 The study proved that the species *V. radiata* and *V. mungo* are, respectively, fluoride-sensitive and tolerant, and it also demonstrated the effectiveness of SNP in reducing stress-induced damage to *Vigna* by enhancing the defence mechanism. The purpose of the study was to investigate the differences in how *Vigna radiata* and *Vigna mungo* responded to fluoride stress (25 mg L^{-1} NaF) and to support the protective effects of sodium nitroprusside.

Yadu et al., 2018 The goal of the study was to track how engineered AgNP (non-dialyzed) helped *Cajanus cajan L*. recover from oxidative damage brought on by fluoride (F). According to experimental findings, F exposure decreased growth and the membrane stability index, although these effects were exacerbated by the addition of AgNP. The investigation shows that atleast in the *Cajanus cajan L*. radicles, AgNP have F-toxicity remediation potential.

Chakrabarti and Patra, 2015 In this study fluoride stress on two rice varieties, *Oryza sativa L.* var. Swarno and *O. sativa L.* var. IR-36, growing in clay pots and

irrigated with aqueous solutions containing 0, 10, 20, and 30 mg NaF/L was examined. Eight widely used biochemical indices, including free amino acids, ascorbic acid, catalase activity, peroxidase activity, chlorophyll, and carotenoid, were calculated. The study found that IR-36 among these rice types was shown to be somewhat more resistant to F stress than the Swarno variety.

Sharma et al., 2020 In this study, a half-diluted Hoagland solution subjected *Vigna radiata L*. to fluoride stress. A concentration-dependent analysis of antioxidant enzyme activity was carried out against the most significant inhibition of growth levels and content of malondialdehyde, the reduce in chlorophyll content reported in seedlings treated by NaF compared to controls.

Singh et al., 2023 The purpose of this study is to investigate the morphological and biochemical alterations in *Brassica juncea (L*.), as well as the variations in its fatty acid (FA) composition and oil output, under two regimes of F^- contaminated soils. Two fluoride contaminated soil regimes were taken to perform the experiment. The amount of F^- in plant tissues (root, leaf, and grain) was substantially greater in Ir_10 (18.3, 14.7, and 2.8, respectively) than in Tr_10 (4.3, 2.6, and 0.77, respectively), but oil yield was significantly lower in Ir_10 (19.5%) than in Tr_10 (44.9%). The phytoremediation capacity of F^- by *Brassica juncea L.* is stronger in the Tr regime than in the Ir regime.

Tyagi et al., 2017 The goal of this research is to determine how F-contaminated soils affect the physiology, growth, and germination of essential pulses including spinach (*Spinacia oleracea*) and mung beans (*Vigna radiata*). This study comes to the conclusion that soil polluted with F negatively affects the biochemical and physiological aspects of mung bean and spinach growth.

Verma et al., 2022 The current study sought to determine the effects on plants of fluoride-contaminated irrigation water (25, 50, 100, and 200 ppm). With increasing fluoride content in irrigation water, growth and biomass attributes (% germination, growth traits, shoot-root length, and fresh-dry mass) decreased. The findings of this study suggest that fluoride-contaminated irrigation water has a negative influence on pea plant seed germination, growth and development, photosynthetic, biochemical, ultrastructural, and biomass.

Zouari et al., 2017 Fluoride toxicity was investigated in young olive plants (*Olea europaea L. cv Chemlali*) that were irrigated with escalating NaF concentrations for 5 months: 0 (control), 20, 40, and 80 mM NaF. The administration of NaF increased the F content in plant tissues, with roots having a higher amount than leaves.

Ahmad et al., 2022 Study investigated the effects of soil F (0, 10, 20, 50, 100, 200 mg kg^{-1}) on wheat morpho-physiological growth characteristics, soil F contents, and bioavailability and uptake of F, phosphorus (P), sulphur (S), potassium (K), calcium (Ca), magnesium (Mg), aluminium (Al), iron (Fe), manganese (Mn), silicon (Si), and zinc (Zn) by wheat. The data revealed increased F absorption and related impairment in nutrient and trace metal build-up, resulting in leaf damage and powdery mildew infection in wheat.

3.OBJECTIVES OF STUDY:

1. To Study Growth and Oxidative Stress Parameters of *Vigna mungo (L.)* genotypes under fluoride stress conditions.
2. Analysis of Superoxide Dismutase (SOD) and Catalase (CAT) Antioxidant Enzyme and Nitrogenase activity of *Vigna mungo (L.)* genotypes at Biochemical Level under fluoride stress conditions.
3. Transcriptional analysis of the antioxidants (SOD and CAT) in selected genotypes of *Vigna mungo (L.)* under fluoride stress.

4.MATERIAL AND METHODS

4.1 Cultivation of Plants:

The five genotypes of *Vigna mungo (L.)* seeds will be surface sterilised with mercuric chloride ($HgCl_2$) (0.1% w/v) before being thoroughly washed with distilled water. The seeds will be put on sterile gauges on each Petri plate after sterilisation, and they will be left to germinate for three days in the dark at 28±2 °C. For an additional seven days, the plates will be kept in a well-ventilated room and subjected to a 260 mol m-2 s-1 photon flux density at 28±2 °C during a 16/8 h light/dark photoperiod cycle**.** The germinated seedlings will be uniformly selected and transferred to plastic pots containing sterile soil. These pots are placed in a thermostatically cultural area-Green house.

4.2 Fluoride treatment and sample harvesting:

Acclimated seedlings of *Vigna mungo (L.)* genotypes will be used for their later experimental work. Fluoride solution nutrient solution at different concentrations will be added. One control pot of all genotypes without fluoride will be used.

4.3 Physiological parameters:

Physiological Parameters recorded will be fresh and dry weight, root and shoot lengths. Fresh weight and dry weight will be recorded (in mg) after Fluoride stress. Additionally measured and depicted in cm scale will be the root and shoot lengths.

4.4 Oxidative Stress-Related Parameters: Electrolyte Leakage, MDA (lipid peroxidation), H_2O_2, Chlorophyll Loss:

The method described by Dionisio-Sese and Tobita (1998) will be used to determine the amount of electrolyte leakage.

The amount of hydrogen peroxide will be measured using a standard curve (Zouari et al., 2017; Sergiev et al.,1997).

The chlorophyll content will be calculated by measuring the absorbance at 645 nm and 663 nm with a UV/VIS double-beam spectrophotometer, expressed as g of chlorophyll g^{-1} of fresh weight (FW) tissue (Singh et al., 2020).

The build-up of reactive 2-thiobarbituric acid (TBA) metabolites, primarily malondialdehyde (MDA), will be used to gauge the degree of lipid peroxidation. The tissues from the roots, stems, and leaves (0.2 g) were extracted in 5 ml of 0.25 percent TBA produced from 10% TCA. 30 minutes of extract heating at 95℃ will be followed by a rapid ice cooling. The supernatant's absorbance will be assessed at 532 nm following centrifugation at 10,000 g for 10 min. By deducting the absorbance measurement made at 600 nm, non-specific turbidity will be corrected. When an extinction coefficient of 155 mm^{-1} cm^{-1} is used, the level of lipid peroxidation is represented as nmol of MDA (Panda and Khan., 2009).

4.5 Biochemical assay for determination of SOD and CAT antioxidant enzymes

a). Extraction of enzymes:

Fresh roots and leaves will be homogenised in an extraction medium containing 0.1 M potassium phosphate buffer, pH 6.8, 0.1M EDTA, 1M phenyl methane sulfonyl fluoride, and 1% polyvinylpyrrolidone in order to measure the activity of antioxidant

enzymes. Centrifuging the homogenate at 12,000 g for 15 min. at 4 °C. The resultant supernatant will be utilised as a crude extract to evaluate the activities of CAT and SOD**.**

b). Enzyme Assays and protein determination:

- The Aebi (1974) assay will be used to measure CAT enzyme activity.
- SOD enzyme activity will be estimated using the assay mentioned in Kono et al., 1979.
- The protein content will be recorded by the method of Lowry et al., (1951).

4.6 Nitrogenase activity

Nitrogen fixation capacity will be measured by acetylene reduction assay (ARA), in situ.

a) Acetylene Reduction Assay (ARA)

By using gas chromatography and a Porapak column, the acetylene reduction assay (ARA), which will be performed in situ and will be used to evaluate the ability to fix nitrogen (Benmoussa et al., 2022).

4.7 Transcriptional level analysis of SOD and CAT enzymes of *Vigna mungo (L.)* at different concentrations of fluoride:

To confirm the amount of SOD and CAT transcription in various regions of *Vigna mungo* (L.), which exhibits a portion of highest reaction, we will conduct semi-quantitative RT-PCR.

First, total RNA will be extracted from 500 mg samples using a mortar and pestle, followed by DNase I free RNase treatment (Promega). Following that, random hexamers and Superscript II reactions will be used to reverse-transcribe the extracted RNA into cDNA in a 20 µl in one reaction. Different *Vigna mungo (L.)* sections will be used for RT-PCR experiments, each with a different fluoride concentration.

For each gene, the number of PCR cycles will be determined in order to produce visible bands on agarose gels. Following that, reverse transcription material will be amplified using primer pair gene-specific PCR. These gene-specific primers will standardise the annealing temperature. To ensure the linearity needed for semi-quantitative RT-PCR analysis, each pair of primers will be amplified using an optimal number of PCR cycles. In order to assess the amount of expression, we will scan 1.2% on stained ethidium bromide gels and analyse the level of SOD, CAT and nitrogenase

mRNA in various plant components at different fluoride concentrations. Their identity was then verified through sequencing.

5. Research questions/Hypothesis

- To observe on which fluoride concentration does *Vigna mungo (L.)* grows best by studying various biochemical and transcriptional level metrices.
- Which one of the five genotypes of *Vigna mungo*, is best suited for fluoride stressed soil of Rajasthan by comparing all the metrices studied.
- Can this legume's nitrogen fixing ability be used to boost crop output under fluoride stress conditions by studying Nitrogenase enzyme activity.

REFERENCES

Aebi H. (1974) Catalases. In: Bergmeyer HU (ed) Methods of enzymatic analysis, Verlag Chemie, Weinheim. Academic Press Inc, New York, p 680.

Agarwal S., Khan S. (2016). Sodium fluoride (NaF) induced changes in growth and DNA profile of Vigna radiata. Plant Sci. Today [Internet]. 2016 Jan. 1 [cited 2022 Nov. 3];3(1):1-5.

Ahmad M., Zahir, Z.A., Asghar H.N. (2012). The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. *Ann Microbiol* **62**, 1321–1330 (2012).

Ahmad M.N., Zia A, den Berg L.V., Ahmad Y., Mahmood R., Dawar K.M., Alam S.S., Riaz M., Ashmore M. (2022) Effects of soil fluoride pollution on wheat growth and biomass production, leaf injury index, powdery mildew infestation and trace metal uptake, Environmental Pollution,Volume 298,2022,118820, ISSN 0269-7491.

Benmoussa S., Nouairi I., Rajhi I., Rezgui S., Manai K., Taamali W., Abbes Z., Zribi K., Brouquisse R., Mhadhbi H. (2022). Growth Performance and Nitrogen Fixing Efficiency of Faba Bean (Vicia faba L.) Genotypes in Symbiosis with Rhizobia under Combined Salinity and Hypoxia Stresses. *Agronomy*, *12*(3), p.606.

Bhattacharya P., & Samal A. (2018). Fluoride contamination in groundwater, soil and cultivated foodstuffs of India and its associated health risks: A review. 7.

Cai H., Yangyang D., Yeyun L., Daxiang L., Chuanyi P., Zhengzhu Z., and Xiaochun W. (2016) "Physiological and cellular responses to fluoride stress in tea (Camellia sinensis) leaves." *Acta physiologiae plantarum* 38, no. 6 (2016): 1-11.

Chakrabarti S., & Patra P. (2015). Biochemical and antioxidant responses of paddy (Oryza sativa L.) to fluoride stress. Fluoride. 48. 56-61.

Dionisio-Sese M.L., Tobita S. (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135(1):1–9.

EPA. (1978). National Air Quality Monitoring and Emissions Trends Rep. 1977. Publ. No. EPA 450/2-78-052, Research Triangle Park, North Carolina.

Gill S.S., Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010 Dec;48(12):909-30.

Gupta B., Pathak G.C., Pandey N. (2011). Induction of oxidative stress and antioxidant responses in *Vigna mungo (L.)* by zinc stress. *Russ J Plant Physiol* 58, 85–91.

Jangir C., Singh D., Kumar S. (2016). "Yield and economic response of biofertilizer and fertility levels on black gram (*Vigna mungo (L.)* L.)." *Prog Rese An Int J* 11. Special-VIII: 5252-5254.

Jat S., Dangi K.L., Kumhar B.L. (2017). Constraints in Adoption of Improved Cultivation Practices of Black Gram.Int.J.Curr.Microbiol.App.Sci. 6(5): 1820-1824.

Jha D.P., Sharma S.K., Amarawat T. (2015) "Effect of organic and inorganic sources of nutrients on yield and economics of blackgram *Vigna mungo (L.)* grown during kharif." *Agricultural Science Digest-A Research Journal* 35.3: 224-228.

Kandukuri S.S, Noor A., Ranjini S.S., Vijaylakshmi M.A. (2012). "Purification and characterization of catalase from sprouted black gram (*Vigna mungo L.*) seeds." *Journal of Chromatography B* 889: 50-54.

Kono Y., Takahashi M., Asada K. (1979). Superoxide dismutases from kidney bean leaves. (1979). *Plant and Cell Physiology*, Volume 20, Issue 7, November 1979, Pages 1229–1235.

Lowry 0.H., Rosebrough N.J., Farr A.L., and Randal R.J. (1951). Protein measurement with the Folin phenol. J. Biol. Chem, 193: 265-275.

Manchanda G and Garg N. (2008). Salinity and its effects on the functional biology of legumes. (2008) *Acta Physiol Plant* 30, 595–618 (2008).

Panda S.K and Khan M.H. (2009). Growth, Oxidative Damage and Antioxidant Responses in Greengram (*Vigna radiata* L.) under Short-term Salinity Stress and its Recovery. Journal of Agronomy and Crop Science, 195: 442-454.

Plá C. L., & Cobos-Porras L. (2015). Salinity: physiological impacts on legume nitrogen fixation. In *Legume nitrogen fixation in a changing environment* (pp. 35-65). Springer, Cham.

Rai A. (2021). "Fluoride contamination issues of groundwater in Rajasthan: a review." (2021).

Rajput A. (2020). Costs and Returns of Mungbean in the Nagaur District of Rajasthan. Economic Affairs. 65. 10.46852/0424-2513.3.2020.5.

Roychoudhury A., Singh A., Aftab T., Ghosal P., Banik, N. (2021). Seedling priming with sodium nitroprusside rescues Vigna radiata from salinity stress-induced oxidative damages. *Journal of Plant Growth Regulation*, 1-11.

Raychaudhuri S.S & Deng X.W. (2000). The role of superoxide dismutase in combating oxidative stress in higher plants. The Botanical Review, 66(1), 89–98.
Rees D. C., & Howard J. B. (2000). Nitrogenase: standing at the crossroads. *Current Opinion in Chemical Biology, 4(5), 559–566.*

Scandalios J.G. (1990). Response of Plant Antioxidant defense genes to Environmental Stress. Advances in Genetics, 1–41.

Schwember A. R., Schulze J., del Pozo A., Cabeza, R. A. (2019). Regulation of Symbiotic Nitrogen Fixation in Legume Root Nodules. *Plants, 8(9), 333.*

Sergiev I., Alexieva V., Karanov E. (1997). Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Competes Rendus del'Academie Bulgare des Sciences 51, 121–124.

Sharma I., & Ahmad P. (2014). Catalase Oxidative Damage to Plants, *131–148.* doi:10.1016/b978-0-12-799963-0.00004-6.

Sharma K., Choudhary M., Verma K. (2020). Role of glutathione reductase and catalase enzyme in antioxidant defense mechanism in controlling fluoride-induced oxidative stress. Journal of Applied Biology and Biotechnology. 2020 Jan 10;8(1):5-8.

Sharma L., and Bhushan B. (2018). Returns from pulses in different regions of Rajasthan at alternative price scenarios. Received: 15-06-2018 Accepted: 15-01-2019 DOI: 10.18805/ag.D-4773.

Sharma R., and Kaur R. (2019). "Fluoride toxicity triggered oxidative stress and the activation of antioxidative defence responses in *Spirodela polyrhiza* L. Schleiden." *Journal of Plant Interactions* 14.1 (2019): 440-452.

Singh G., Kumari B., Kriti Sinam G., Kulsoom M., Niranjan A., Patnaik S., Chauhan P.S., Mallick S. (2023) Changes in fatty acids in Brassica juncea L. oil grown under two simulated conditions of fluoride contamination. International Journal of Phytoremediation. 2023 Apr 18:1-8.

Singh A., Banerjee A., Roychoudhury A. (2021). Differential Responses of *Vigna radiata* and *Vigna mungo (L.)* to Fluoride-Induced Oxidative Stress and Amelioration via Exogenous Application of Sodium Nitroprusside. *J Plant Growth Regul* 40, 2342–2357.

Singh A., Roychoudhury A. (2020). Silicon-regulated antioxidant and osmolyte defense and methylglyoxal detoxification functions co-ordinately in attenuating fluoride toxicity and conferring protection to rice seedlings. Plant Physiol Biochem. 2020 Sep;154:758-769.

Singh M., Verma K.K., Verma C.L. (2013). An approach to develop a model for describing the influence of fluoride-contaminated irrigation water on physiological responses in poplar (*Populus deltoides* clone S_7C_{15}). *Acta Physiol Plant* 35, 3357–3364.

Tyagi R., Luhach J., Mishra A.K., Chaudhry S. (2017). Morphological and biochemical changes in Vigna radiata and Spinacia oleracea induced by fluoride contamination in soils. International Journal of Current Microbiology and Applied Sciences. 2017;6(5):395-402.

Verma K.K., Verma P., Singh M. (2022) Influence of fluoride phytotoxicity in germinating seedlings of *Pisum sativum*: modeling of morpho-physiological traits. *Vegetos* **35**, 736–746.

Yadu B., Chandrakar V., Korram J., Satnami M.L., Kumar M., SK. (2018). Silver nanoparticle modulates gene expressions, glyoxalase system and oxidative stress markers in fluoride stressed Cajanus cajan L. Journal of Hazardous Materials, *353, 44–52.*

Yadu B., Chandrakar V., Sahu K. (2016). Responses of plants to fluoride: An overview of oxidative stress and defense mechanisms. Fluoride. 49. 293-302.

Zouari M., Elloumi N., Bellassoued K., Ahmed C.B., Krayem M., Delmail D., Elfeki A., Rouina B.B., Abdallah F.B., Labrousse P. (2017). Enzymatic antioxidant responses and mineral status in roots and leaves of olive plants subjected to fluoride stress, South African Journal of Botany, Volume 111, 2017, Pages 44-49, ISSN 0254-6299.